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THE PRODUCTION OF ANTISERUM FOR AGGLUTINATION TESTS

Francois H. K. Reynolds and J. F. Hill
From the Laboratory of the Southeastern Department, Fort McPherson, Ga.

On subcutaneous and intraperitoneal inoculation of organisms, washed from agar slants and killed by heating, death of the rabbits often occurred, especially in the cases of B. enteritidis (Gärtner), paracolon and paratyphoid bacilli, and when the animal survived, repeated inoculations were required to produce a serum of satisfactory titer. The marked toxicity of the killed suspensions led us to try to overcome this troublesome feature, and the results are given briefly.

Growths from agar cultures were treated as before, the suspensions being killed in a water bath at 60 C. for 30 minutes, after which that portion of the tube above the liquid was flamed in order to destroy any organisms which might have survived, as the tube was not totally submerged. Cultures were then made to ascertain definitely the absence of living organisms.

TABLE 1

Result of Inoculation of Rabbits

Rabbits	Suspension	Inoculations	Amounts in C C	Dates Killed	Final Titers
61-A	B. enteritidis 1	3/11	1.5		
		3/26	2	4/10	1:10,000
10	B. paratyphosus "A"	4/23	2	5/ 9	1:10,000
760	B. enteritidis 1	3/20	2	4/10	1:6,600
756	B. paracolon	4/14	1	4/29	1:6,600
421	B. paracolon	4/14	1		
		4/28	2	5/12	1:10,000
5	B. enteritidis 1	4/23	2		
		5/ 1	2	5/15	1:10,000
6	B. enteritidis 1	4/23	3		
		5/ 1	2	5/15	1:5,000
7	B. enteritidis 104-B	4/23	2		
		5/ 1	2	5/15	1:5,000

The following day the suspensions received at least three washings in sterile salt solution; the supernatant fluid being poured off and replaced each time with fresh salt solution; the clumps were disintegrated and the organisms distributed evenly throughout the suspensions by agitating the tube. After the third washing, salt solution was added and the suspensions filtered through cotton, the density being regulated by comparison with a nephelometer. These suspensions were inoculated in the ear vein under antiseptic precautions. The animals showed no apparent discomfort and the table illustrates the efficacy of this cell-washing method. In one instance, the first injection produced a titer of 1 to 10,000, and the results as a whole are far better than those of the older method.

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The bacterial suspensions used were washed from a 24-hour agar slant with carbolized (0.5%) salt solution, filtered through cotton and standardized by a nephelometer of such a density that the graduations on a 5 c c pipet held in the middle of a test tube of the fluid are just visible. The immune serum was made up in dilutions of 1 : 10, 1 : 100 and 1 : 1,000, with salt solution, and varying quantities used to give the desired range of concentrations in the test. In addition to the serum each tube contained 0.25 c c of salt solution and 0.2 c c of the bacterial suspension. The mixtures were incubated at 37.5 C. in a water bath for 24 hours and read. In positive mixtures there was a clear fluid and a characteristic "scattering" sediment with an irregular border, markedly different from the negative tubes even before the tubes were agitated to reveal the clumping.

It appears that washing rids the suspensions of certain toxic materials and facilitates the production of antiserum. It is possible that the method may be of advantage in the case of other organisms as well and in the fact that more marked agglutination is produced than when larger quantities are used.